

CLAIMS

The following represents the current status of all the claims in the present application including changes made by this paper:

1-134(canceled).

135(previously presented). An in vitro method of incorporating at least one polynucleotide encoding a desired trait into a male germ cell, comprising:

- (a) obtaining a male germ cell from a non-human vertebrate species, said germ cell being selected from the group consisting of spermatogonial stem cells, type B spermatogonia, primary spermatocytes, preleptotene spermatocytes, leptotene spermatocytes, zygotene spermatocytes, pachytene spermatocytes, secondary spermatocytes, spermatids, and spermatozoa;
- (b) transfecting the germ cell in vitro with at least one polynucleotide encoding a gene product in operable linkage with a promoter comprised in a virus or virus-derived DNA, in the presence of a gene delivery mixture comprising at least one transfecting agent, and optionally a polynucleotide encoding a genetic selection marker;
- (c) allowing the polynucleotide encoding a gene product to be taken up by, and released into the germ cell; and

(d) selecting those cells in which the polynucleotide has incorporated into the genome of the germ cell.

136(canceled).

137(previously presented). The method of claim 135, wherein the male germ cell is selected from the group consisting of spermatogonial cells and other undifferentiated male germ cells.

138(previously presented). The method of claim 135, wherein the transfection is conducted under conditions of temperature of about 25°C to about 38°C.

139(canceled).

140(previously presented). The method of claim 135, wherein the viral vector is selected from the group consisting of retroviral vectors, adenoviral vectors, transferrin-polylysine enhanced adenoviral vectors, Moloney murine leukemia virus-derived vectors, mumps vectors, and virus-derived DNAs that enhance polynucleotide uptake by and release into the cytoplasm of germ cells or a mixture of any members of said group.

141(previously presented). The method of claim 140, wherein the retroviral vector is selected from the group consisting of lentiviral vectors.

142(previously presented). The method of claim 135, wherein the transfecting agent comprises an adenovirus vector having endosomal lytic activity, and the polynucleotide encoding

a gene product is operatively linked to the vector.

143(previously presented). The method of claim 135, wherein the polynucleotide encoding a gene product is in the form of a complex with a viral vector.

144(canceled).

145(previously presented). The method of claim 135, wherein:

the transfecting agent further comprises an agent selected from the group consisting of a c-kit ligand and at least one genetic selection marker; and

the method further comprises isolating or selecting a male germ cell carrying at least one polynucleotide encoding a gene product at least one polynucleotide encoding a genetic selection marker, from a donor male vertebrate with the aid of the genetic selection marker.

146(previously presented). The method of claim 145, wherein the genetic selection marker comprises a gene expressing a detectable product, driven by a promoter selected from the group consisting of c-kit promoters, b-Myb promoters, c-raf-1 promoters, ATM (ataxia-telangiectasia) promoters, RBM (ribosome binding motif) promoters, DAZ (deleted in azoospermia) promoters, XRCC-1 promoters, HSP 90 (heat shock gene) promoters, and FRMI (from fragile X site) promoters.

147(previously presented). The method of claim 135,

wherein the non-human vertebrate is a mammal.

148(previously presented). The method of claim 147, wherein the mammal is selected from the group consisting of non-human primates and farm and marine mammals.

149(previously presented). The method of claim 135, wherein the polynucleotide encoding a gene product is derived from the same non-human vertebrate species as the germ cell.

150(previously presented). The method of claim 135, wherein the non-human vertebrate is selected from the group consisting of wild and domesticated vertebrates.

151(previously presented). The method of claim 135, wherein the polynucleotide encoding a gene product is derived from a non-human mammal selected from the group consisting of human and non-human primates, canines, felines, swines, farm mammals, pachyderms, marine mammals, equines, murines, ovines and bovines, or from a bird selected from the group consisting of ducks, geese, turkeys and chickens.

152(previously presented). The method of claim 151, wherein the polynucleotide is derived from a human.

153(previously presented). The method of claim 135, wherein the promoter is a germ cell-specific promoter.

154(previously presented). The method of claim 135, wherein the polynucleotide encoding a genetic selection marker is operatively linked to a germ cell-specific promoter.

155(previously presented). The method of claim 145, wherein the polynucleotide encoding a genetic selection marker is operatively linked to a germ cell-specific promoter.

156(previously presented). The method of claim 135 including a further step of introducing transfected cells selected in step (d) into a testis of a male of a non-human vertebrate of the species from which said male germ cell was obtained, thereby producing injected males.

157(previously presented). The method of claim 156 wherein said transfected cells are injected into said testis via vasa efferentia.

158(previously presented). The method of claim 156 including a further step of breeding one or more of said injected males to one or more normal females to thereby produce transgenic non-human mammal progeny.

159(previously presented). A transgenic non-human mammal produced by the method of claim 158.

160(previously presented). The method of claim 135 wherein said at least one polynucleotide encoding a gene product in step (b) is one which is able to correct a gene disorder.